# (12) UK Patent Application (19) GB (11) 2 270 752 (13) A

(43) Date of A Publication 23.03.1994

(21) Application No 9219628.6

(22) Date of Filing 16.09.1992

(71) Applicant(s)
**Azim Fazal**
**13 Shere Avenue, CHEAM, Surrey, SM2 7JX,**
**United Kingdom**

(72) Inventor(s)
**Azim Fazal**

(74) Agent and/or Address for Service
**Brookes & Martin**
**High Holborn House, 52-54 High Holborn, LONDON,**
**WC1V 6SE, United Kingdom**

(51) INT CL$^5$
**G01N 33/48, C12Q 1/04**

(52) UK CL (Edition M)
**G1B BBE**

(56) Documents Cited
**WO 91/19974 A1**

(58) Field of Search
**UK CL (Edition L) G1B BBD BBE**
**INT CL$^5$ C12Q, G01N**
**ONLINE DATABASES:**
**WPI,CLAIMS,JAPIO,ANALYTICAL ABS.,CAS ONLINE.**

## (54) Diagnosing parasitic diseases

(57) Parasitic diseases of the blood, e.g. malaria, are diagnosed by centrifuging a blood sample to separate the erythrocytes, lysing the erythrocytes to release any parasites within the erythrocytes, and counting the number of parasites in the lysed sample. Process is capable of automated operation.

The claims were filed later than the filing date within the period prescribed by Rule 25(1) of the Patents Rules 1990.

# DIAGNOSTIC METHOD

This invention is concerned with a method for diagnosing parasitic diseases of the blood, especially malaria and in particular provides a method susceptible to automated operation in a laboratory.

Malaria is a predominantly African problem where it annually kills or debilitates millions of people. The mainstay of laboratory diagnosis is the finding of malaria parasites in stained films of blood taken from suspected patients. This method introduces human error, which is not negligible, and thus results in doubts as to the sensitivity of the results. The World Health Organisation recommends looking at the stained thick blood film for three to five minutes - a completely unrealistic and impractical suggestion in view of the workload in most developing countries.

Accordingly there is a need for a diagnostic system which allows a non-subjective assessment of results and is preferably capable of automated operation.

The present invention provides a diagnostic method which comprises centrifuging a blood sample to separate the erythrocytes, lysing the erythrocytes to release any parasites within the erythrocytes, and counting the number of parasites in the lysed sample.

Normally the blood sample will be treated with an anticoagulant in conventional manner before centrifugation. After centrifugation, plasma and other blood components can be removed to isolate the erythrocytes (red blood cells). A measured aliqu of the red cells is removed. These cells are then lysed, for example physically using ultrasonics or chemically using haemolytic agents.

The lysis is controlled, for example by adjustment of frequency or power of an ultrasonic transducer, or by adjustment of concentration of a haemolytic reagent, so as to release intra-erythrocyte parasites, such as malaria parasites, intact. The parasites can then be counted using conventional cell counting techniques. The use of electronic counters means that an objective assessment of the parasite population can be made. By appropriate selection of the size of the red cell sample, a larger population of parasites is available for counting, increasing the accuracy.

Malaria parasites are of a size that can be easily distinguished by conventional cell counters in a solution of haemoglobin and other disintegration products of the lysed red cells. For example the parasites of the Falciparum species may be from 2-5 microns in size.

The centrifuging, lysing and counting operation are readily susceptible to automated operation, allowing rapid and objective assessment of blood samples.

The invention has been described above in terms of detecting intra-erythrocyte parasites. Clearly the technique is also applicable to parasitic infestations of other separable blood components if needed.

CLAIMS:

1. A method for diagnosing parasitic diseases of the blood comprising:

centrifuging a blood sample to separate the erythrocytes;

lysing the erythrocytes to release any parasites within the erythrocytes; and

counting the number of parasites in the lysed sample.

2. A method as claimed in Claim 1, wherein before centrifugation the blood sample is treated with an anticoagulant.

3. A method as claimed in Claims 1 or 2, wherein the sample is lysed using ultrasonics.

4. A method as claimed in Claims 1 or 2, wherein the sample is lysed chemically using haemolytic agents.

5. A method as claimed in any one of the preceding claims, wherein the parasites are malaria parasites.

**Patents Act 1977**
**Examiner's report to the Comptroller under Section 17**
**(The Search report)**

Application number
GB 9219628.6

**Relevant Technical Fields**

(i) UK Cl (Ed.L) G1B (BBD, BBE)

(ii) Int Cl (Ed.5) G01N, C12Q

Search Examiner
M R WENDT

Date of completion of Search
22 OCTOBER 1993

**Databases (see below)**
(i) UK Patent Office collections of GB, EP, WO and US patent specifications.

(ii) ONLINE DATABASES: WPI, CLAIMS, JAPIO, ANALYTICAL ABS, CAS ONLINE

Documents considered relevant following a search in respect of Claims :-
1-5

**Categories of documents**

**X:** Document indicating lack of novelty or of inventive step.

**Y:** Document indicating lack of inventive step if combined with one or more other documents of the same category.

**A:** Document indicating technological background and/or state of the art.

**P:** Document published on or after the declared priority date but before the filing date of the present application.

**E:** Patent document published on or after, but with priority date earlier than, the filing date of the present application.

**&:** Member of the same patent family; corresponding document.

| Category | Identity of document and relevant passages | Relevant to claim(s) |
|---|---|---|
| A | WO 91/19974 A1 (FLOW INC) See Claims 1 and 2 | 1 |

**Databases:** The UK Patent Office database comprises classified collections of GB, EP, WO and US patent specifications as outlined periodically in the Official Journal (Patents). The on-line databases considered for search are also listed periodically in the Official Journal (Patents).